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The evolution of floral guides: using a genetic algorithm to investigate the evolution of floral cue arrangements

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ABSTRACT

Many floral displays incorporate complex contrasting stimuli patterns in visual, olfactory and other sensory modalities. These 'floral guides' can increase pollen transfer rates and visitation rates for flowers while reducing the search times of pollinators. Although these guides are important in mediating the relationship between plants and pollinators, little is known about the evolution of these guides or the conditions needed for them to evolve. Here we use a novel approach by applying a genetic algorithm, a modelling method inspired by biological evolution, to investigate floral guide evolution. Specifically, we examine the ways in which pollinator behaviour can affect the evolution of radiating floral guides and what conditions are necessary for this development. The results suggest that flower size and pollinator directionality, starting location and movement type affects the development of radiating guides. A preference for cues over a lack of cue is also necessary for this development. These findings suggest that radiating floral guides can evolve independently of specific perceptual biases by the pollinator other than a preference for cues over their absence. Despite these findings, we are aware of the limitations of computational models, and hope that these findings inform and motivate future models and empirical studies.

Additional Keywords: Floral displays, coevolution, radiating guides, plant-pollinator interactions

INTRODUCTION

Flowering plants rely on pollinators to enhance reproduction through the transfer of pollen between conspecifics using the pollinator as a vector (Crepet, 2008), and have developed many signalling methods to enhance the pollinators' ability to accurately locate the flower and its associated rewards (Daumer, 1958; Free, 1970; Wacht, Lunau, & Hansen, 1996; Kelber, 1997; Dinkel & Lunau, 2001; Kelber, Balkenius, & Warrant, 2002; Raguso, 2004a, 2008; Goyret, Markwell, & Raguso, 2007; Leonard & Papaj, 2011). One taxonomically widespread feature of flowers are floral guides, which are spatial cues that aid in close-up orientation towards floral rewards (Hansen, Van der Niet, & Johnson, 2012). Out of 41 randomly selected wild and cultivated flowers, 36 had these guides (Penny, 1983), highlighting their pervasiveness. These floral guides, first described by Sprengel (1793), are often spots, lines and blotches of contrasting colour on the petals which radiate from the nectary of the flower (Manning, 1956; Daumer, 1958; Kugler, 1963; Faegri & Van der Pijl, 1979). As nectarivore survival and reproductive success relates to nectar collection rate (Pelletier & McNeil, 2003; Burns, 2005), flower visitors can reduce flower handling times and maximise their nectar collection rate through the use of these floral guides (Leonard & Papaj, 2011; Hansen *et al.*, 2012; Goodale *et al.*, 2014; Lawson, Whitney, & Rands, 2017a). Although the study of floral guides has focused on their use by bees, the use of, or preference for, these guides has been observed in flies, butterflies, hummingbirds and moths (Knoll, 1922; Dinkel & Lunau, 2001; Medel, Botto-Mahan, & Kalin-Arroyo, 2003; Hansen *et al.*, 2012). Spatial fragrance patterns, whereby there are uneven distributions of scent across the floral surface, may also reduce search times on flowers (Lawson *et al.*, 2017a) and similar temperature patterns could also exist (Harrap *et al.*, 2017). This reduced handling time is a clear benefit to a foraging pollinator but it could also benefit the plant by increasing pollen transfer rates (Leonard & Papaj, 2011). It has also been suggested that floral guides prevent pollen-theft behaviour by floral visitors, conveying an additional benefit to the plant (Ushimaru, Watanabe, & Nakata, 2007). Floral guides are also thought to be under strong selective maintenance, for example, the experimental removal of floral guides in the iris *Lapeirousia oreogena* significantly reduced both pollen export and fruit set (Hansen et al. 2012).

Pollinators can show strong preferences for plants with these patterns. Both hummingbirds (*Salasphorus platycercus* and *S. rufus*) and bumblebees (*Bombus appositus, B. nevadensis* and *B. flavifrons*) show a preference for visually patterned flowers of montane larkspur (*Delphinium nelsonii*) compared to their albino morphs (Waser & Price, 1985). It is possible that these guides may also act to reinforce flower constancy, whereby foragers preferentially visit flowers of a single species (Waser, 1986), as these guides could act as an additional trait which can be used to distinguish flowers (West & Laverty, 1998; Fauria *et al.*, 2002). Floral guides can also increase the relative frequency of legitimate visits by decreasing nectar robbing on flowers which are at risk (Leonard *et*

al., 2013), highlighting how the presence of floral guides can have direct benefits to plant fitness. Guides often incorporate lines which radiate from the nectary (Faegri & Van der Pijl, 1979; Proctor, Yeo, & Lack, 1996) which may have evolved due to pre-existing sensory biases of insects which have developed to enhance behaviours related to returning to nest burrows (Biesmeijer *et al.*, 2005). Many bee-pollinated flowers such as *Iris, Salvia* and *Ipomoea* present UV-reflective radiating lines (Kugler, 1935; Daumer, 1958; Silberglied, 1979; Leonard & Papaj, 2011), which is perhaps unsurprising as bees are known to prefer patterns containing radiating elements (Lehrer *et al.*, 1995). These patterns also tend to contrast against other parts of the flower (Penny, 1983; Hempel de Ibarra & Vorobyev, 2009). It is therefore likely that these patterns evolved to exploit the pre-existing sensory biases of pollinators, such as preferences for contrasting cues.

Little is currently known about the evolution of these patterns. Although fossilised flowers have made substantial contributions to our understanding of floral evolution (Crepet, Nixon, & Gandolfo, 2004), there is currently no fossil evidence shedding light on scent or pigment pattern evolution (Bannister et al. 2005). Considering this lack of current knowledge and the opportunity floral displays present in terms of signal evolution, new techniques must be employed to explore this evolutionary development. Here, we aim to explore the process of floral pattern evolution in a population of simulated flowers using a simple genetic algorithm (Holland, 1975; Goldberg & Holland, 1988), in which flower fitness is linked to the number of movements a pollinator needs to reach the nectary. We examine the different ways in which pollinator behaviour can affect the development of radiating guides on flowers and what conditions must be in place for this development. In particular, we explore the effects of different degrees of visitor directionality, their starting location, movement type and size of flowers on the evolution of floral patterns. Exploring this floral signal complexity provides a unique opportunity to gain insights into signal evolution, the relationship shared between plants and their animal pollinators and the selective maintenance of these relationships.

METHODS

Overview of model

The genetic algorithm simulates the evolution of consecutive generations of flowers in a population, where each flower is described by a single chromosome that phenotypically translates to a grid of non-overlapping uniform square cells that cover the entire surface of the flower (Fig. 1). Flowers are assumed to be square of width *w* cells, and each flower has a nectary in the central cell. Each chromosome codes for the total floral surface of a single flower with each gene coding for a single cell on the square grid. Within an individual, these genes can have one of two alleles: blanks (0s), or cues (1s). The single exception to this is the gene coding for a nectary, which occurs at the centre of the flower. The position of the nectary gene does not change between individuals or generations.

At the beginning of a simulation, the model considers a population consisting of 1000 individuals with entirely blank flowers (all non-nectary genes are coded as blanks). Having initialised the population, selection processes begin by evaluating the fitness of individuals based upon the time it takes a number of pollinators to locate the nectary on an individual flower, given that they land at a randomly defined point on the flower and are predisposed to following cues. Flowers with the highest fitness are those where pollinators spend the smallest amount of time travelling to the nectary once they have landed. Having evaluated the fitness of all individuals in a generation, they are then allowed to breed (where fitness dictates the probability of breeding) and produce offspring that contribute to the next generation, with gene mutation introducing novel heritable patterns of possible cues in offspring that could influence their own fitness. This process is repeated for 150,000 generations.

Calculating floral fitness

The fitness of an individual flower is calculated by simulating the behaviour of three visiting pollinators, and using these visits to generate a metric related to the time it takes them to discover the flower's central nectary. During a visit, a pollinator initially lands in a randomly selected cell. Models were considered where the pollinator could land with equal chance in any of the cells that were not the nectary, or where the pollinator could land with equal chance on any of the perimeter cells on the four edges of the flower. On landing, the pollinator's initial direction of movement is randomly

allocated, with equal chance of facing in either the four cardinal directions, or the eight cardinal and ordinal directions dependent upon the pollinator's movement type.

Once position and direction have been allocated, the pollinator then conducts a series of consecutive movements, ending at the movement where it reaches the nectary. In a movement, the pollinators were assumed to follow one of two movement types, and either move to the four (or fewer) adjacent cells that shared a side with the current cell, or the eight (or fewer) cells that shared either a side or vertex – this choice of movement type was fixed for a given model. If the four or eight neighbouring cells contained a mixture of blank cells and cells containing cues, the pollinator chooses to move to a cell containing a cue with probability $nc/(nc+(b-n))$, where b is the number of neighbouring cells (which is either 8 or 4 dependent on movement type when the pollinator is not at the edge of the flower, but which will be lower when the pollinator is at the edge or a corner), n is the number of neighbouring cells that contain cues, and c is a cue preference parameter. If all available neighbouring cells are identical, the pollinator's movement preference is set at whatever the cells contain.

If at any time an available neighbouring cell contains the nectary, the pollinator is assumed to move directly to the nectary as its next and final movement. Otherwise, if the pollinator can only move to four neighbouring cells, these cells are allocated weightings dependent upon the pollinator's orientation. If $y(\theta)$ is the weighting of the cell that the pollinator is facing when it rotates θ^o from its current orientation, then we assume that $y(180) = 1$, $y(\pm 90) = d$ and $y(0) = d^2$, where d is a pollinator movement directionality parameter. If a pollinator can move to eight neighbouring cells, these are weighted at $y(0) = d^4$, $y(\pm 45) = d^3$, $y(\pm 90) = d^2$, $y(\pm 135) = d$, and $y(180) = 1$. If the presence or absence of cues in the neighbouring cells is similarly denoted as $q(\theta)$ where $q(\theta)=1$ when a cue is present and $q(\theta)=0$ when a cue is absent, then we can calculate the summed neighbour weight of cells with cues as $s_{cue} = \Sigma q(\theta)y(\theta)$ assuming summing over all the neighbouring cells. Similarly, the summed neighbour weight of blank cells is $s_{blank} = \Sigma(1-q(\theta))y(\theta)$. If the pollinator has chosen to move to a cell containing a cue, its probability of rotating θ^o and moving to the cell directly in front of it is $s_{cue}.q(\theta)$. Similarly, if the pollinator has chosen to move to a blank cell, its probability of rotating θ^o and moving directly forwards is $s_{blank}.(1-q(\theta))$. Once a pollinator has moved, its orientation is updated where it was assumed to now be facing away from the cell it has just moved from. Within the simulated environment, this movement type manifests as a 'correlated random walk' (Kareiva & Shigesada, 1983), which is used frequently to model the movements of animals, particularly insects (Wiens, Crist, & Milne, 1993; Bergman, Schaefer, & Luttich, 2000; Byers, 2016).

Each flower i in the population has the movement of three independent pollinators simulated on it. If m_j is the number of movements pollinator j requires to reach the nectary during a simulation and F_{max} is an arbitrary maximum fitness based on the size of the flower (taken to be $15w$), then the fitness of flower i is calculated as $F_i = \Sigma\max(0,F_{max} - m_j)/3$, where the summation is over all three pollinators. Therefore, flowers requiring fewer movements to reach the nectary have the higher fitness values.

Reproduction and selection

Parent flowers are selected using a fitness proportional roulette-wheel selection (Eiben & Smith, 2007), whereby flowers with higher fitness values are more likely to be chosen. We assume that the population consists of I_{max} individuals, each consecutively numbered from between 1 and I_{max}. The total fitness of the population is calculated as $F_{total} = \Sigma F_i$ over all individuals in the population.

Each individual i has a running summed fitness t_i sequentially allocated to it, such that $t_1 = F_1$, and $t_{i>1} = t_{i-1} + F_i$. An individual is selected for breeding by generating a random integer from the range $r = [1, F_{total}]$ where all values are equally likely, and selecting the individual b such that either $t_1 \geq r$, or $t_b \geq r$ and $t_{b-1} < r$. Two individuals are independently selected in this manner in order to breed, indicated here as $parent_1$ and $parent_2$.

To breed, a random gene g was selected from $(0, 1, \ldots, w^2)$, where all values are equally likely. Individual genes from $parent_1$ are copied in ascending sequence to the offspring until gene g is reached, after which subsequent genes are copied from $parent_2$. As genes are copied they have probability 0.0001 of mutating (ignoring the central nectary, which is fixed as a nectary throughout the model). If a gene mutates, it has an equal chance of becoming either a blank or a cue, meaning that half of the mutations experienced by a flower were silent. The nectary never mutates during the crossover process.

Breeding is repeated until 1000 novel offspring are generated. These offspring are assumed to form the next generation, and the current generation is then removed from the simulation.

Model exploration

To explore the model, we manipulated the following parameters between simulations:

- **flower size**, where three types were considered with w = (11 by 11 cells (size 11), 21 by 21 cells (size 21), 31 by 31 cells (size 31));

- **pollinator starting location**, where two types were considered, where pollinators either started by landing randomly in a cell anywhere on the flower (except the nectary), or landing randomly in a cell on the periphery of the flower. The decision was made to incorporate two starting locations as flower visitors have been observed landing at various points on flowers in previous work (Free, 1970);
- **pollinator movement type**, where two types were considered, where the pollinator could move in four directions (to all immediate neighbouring cells not including those diagonally touching) or eight directions (including diagonal neighbours);
- **pollinator movement directionality probability**, where seven types were considered with d = (1.0, 1.5, …, 4.0); and
- **cue preference**, where three types were considered with c = (2, 6, 10).

The combination of these five manipulated parameters led to the simulation of 252 different permutations. 84 additional simulations were generated where the cue preference value is c = 1, and all possible sets of the other four parameters were simulated as described above.

To assess model performance, we collected summary statistics from each simulation. We calculated the mean fitness of all 1000 individuals in the population. We also randomly selected 100 sample flowers from the population and calculated:

- **total cue count** – Mean sum of the total number of cues from sample flowers.
- **cue connection value** – Cue cells which were connected to the nectary via other cues were assigned values based on the minimum number of movements it would take to reach the nectary via connected cues (Fig. 2). These values were then divided by the number of cues on that flower generating an average. The sum of these means from the 100 sample flowers was then divided by 100, giving the mean cue connection value.
- **lonely cue count** – The sum of the total number of cues in the sample flowers which were not connected to the nectary via other cues (cells illustrated with an ‘x’ in Fig. 2).

These statistics were calculated for each generation. For analysis, the results of a simulation was represented by the mean value of each of these summary statistics calculated during each of the 150,000 generations.

Results focussed on the relationship and effects of different parameters and values with cue connection value and fitness, which was used to measure to what degree floral cue arrangements radiated from the centre. All data were analysed using nonparametric tests as the data did not fit the

requirements for parametric testing. Correlations between mean cue connection value and mean fitness, mean total number of cues and mean lonely cue counts were analysed using Spearman's rank correlation. The effect of starting location and movement type of the pollinator on cue connection value was analysed using paired Wilcoxon signed rank tests. Kruskal-Wallis rank sum tests were used to analyse the effects of directionality, flower size and cue preference value followed by *post hoc* analysis in directionality and flower size analysis using Dunn's tests with Holm-Bonferroni corrections to avoid familywise errors for those which merited further investigation. A visual analysis of artificial flowers with Cue preference values at 1, whereby cue cells and blank cells have equal probabilities of being moved to, was also conducted. We are aware that this lack of preference in movement does not align with our current knowledge on insect vision and movement (Floreano *et al.*, 2004; Egelhaaf *et al.*, 2012), however, this was done in order to see if radiating patterns developed without a preference towards cues.

All computations were conducted using the programming language C in Code::Blocks 13.12, SDK Version 1.19.0 using an Intel® Core™ i3-3220T CPU @ 2.80GHz Processor, Windows 7 Operating System and 4.00 GB RAM. Stochastic elements are coded using the *Mersenne Twister* pseudorandom number generator (Matsumoto & Nishimura, 1998). Sample code is provided in the Supplementary Information.

RESULTS

Development of floral guides

Radiating guides developed, to varying degrees, in all 252 simulations over the 150,000 generations. Floral cues were selected for in cells close to the nectary in early generations, with developing guides extending outwards from the nectary in later generations (Fig. 3A). Eventually, patterns reach a point in their development where the small changes over time would not affect the fitness or cue connection values (Fig. S1). This demonstrates that radiating cue arrangements can develop in populations of simulated flowers where fitness is linked to the number of movements a pollinator needs to reach the nectary.

Flower size

There was a negative correlation between mean flower fitness and mean cue connection value for size 11 flowers ($r_s = 0.216$, $p = 0.048$) and a positive correlation for flower sizes 21 and 31 ($r_s = 482$, $p < 0.0001$, $r_s = 0.628$, $p < 0.0001$ respectively) (Fig. 4). Mean cue connection value also increased considerably with the size of flowers (Fig. 5) (Kruskal-Wallis test: $\chi^2_2 = 220.27$, $p < 0.0001$). Fitness also increased with size of flower (Fig. 5) (Kruskal-Wallis test: $\chi^2_2 = 134.11$, $p < 0.0001$).

Pollinator starting location

Flowers with pollinators starting at the perimeter had higher mean cue connection values than those where pollinators started in random cells on the flower, suggesting that flowers where pollinators that landed on the edge of the flower had higher degrees of radiating elements (paired Wilcoxon signed rank test: random starting point: 6.55 ± 2.34 cue connection value (mean ± SD); edge starting point: M = 6.69 ± 2.38 cue connection value, W = 7030, p < 0.0001). However, mean fitness was lower when pollinators started at the edge (paired Wilcoxon signed rank test: random starting point: 218.27 ± 70.43 fitness value; edge starting point: 204.25 ± 67.50 fitness value, W = 124, p < 0.0001), presumably because the mean number of movements was higher when forced to start at the furthest possible distance from the nectary.

Pollinator movement type

Flowers with pollinators that could move in four directions had higher mean cue connection values than those where eight directions were possible (paired Wilcoxon signed rank test: 4-way movement: 6.68 ± 2.31 cue connection value; 8-way movement: 6.57 ± 2.41 cue connection value, W = 5782, p < 0.0001, N = 252). Mean fitness was lower when pollinators were constrained to move in four directions (paired Wilcoxon signed rank test: 4-way movement: 189.47 ± 57.01 fitness value; 8-way movement: 233.06 ± 73.54 fitness value, W = 0, p < 0.0001), again presumably because average journey distance was longer when movement was restricted.

Pollinator movement directionality

Both mean cue connection value and fitness increase with pollinator movement directionality (Fig. 6) (cue connection: Kruskal-Wallis test: $\chi^2_6 = 23.21$, $p = 0.0007$; fitness: Kruskal-Wallis test: $\chi^2_6 = 24.48$, $p = 0.0004$). This is demonstrated in Fig. 3A where the example flowers with lower directionalities develop cue arrangements which are more grouped around the nectary than those with higher directionalities where cues branch out from the nectary.

Pollinator cue preference

Pollinator cue preference had no effect on mean cue connection value (Kruskal-Wallis test: $\chi^2_2 = 0.1768$, $p = 0.915$) (Fig. 7). This suggests that the probability of a pollinator visiting a cue cell over a blank cell did not influence the eventual development of radiating elements. However, additional simulations where cue preference value was reduced to 1 (where there is equal likelihood of visiting a blank cell or a cell with a cue), were undertaken but did not result in the formation of flowers with floral guides (Fig. 3B). This suggests that once there is a degree of preference for cues over blank cells then radiating elements will develop. These additional simulations were not included in the analysis as the flowers generated were entirely random arrangements of cues (Fig. 3B) which only served to add noise to the mean values. However, cue preference value did affect mean fitness (Kruskal-Wallis test: $\chi^2_2 = 36.35$, $p < 0.0001$) (Fig. 7).

Lonely cue count

The relationship between mean lonely cue count and mean cue connection value revealed a complex relationship which was affected by the size of the flower (Fig. 8), where there was a slight negative correlation in flowers of size 11 (r_s = -0.262, p = 0.016) and a strong negative correlation in flowers of size 21 (r_s = -0.622, p < 0.001) and size 31 (r_s = -0.756, p < 0.001).

Mean total cue count

The relationship between mean total cue count and mean cue connection value (Fig. 9) showed a positive correlation with all tested flower sizes (Flower size 11: r_s = 0.398, p < 0.0002; Flower size 21: r_s = 0.515, p < 0.0001; Flower size 31: r_s = 0.402, p < 0.0001). There was also a relationship between mean total cue count and mean fitness, but only with size 21 flowers (Fig. 9). Mean fitness and mean total cue count demonstrated no correlation in flowers of size 11 (r_s = 0.183, p = 0.096), a negative correlation in flowers of size 21 (r_s = 0.354, p = 0.001) and no correlation in flowers of size 31 (r_s = 0.104, p = 0.344).

DISCUSSION

Floral displays are a key feature of the evolutionary success of flowering plants as these signals mediate the interactions between these plants and their animal pollinators. Here, we find that both floral size, and pollinator movement behaviour and preference for cues influences the degree to which radiating patterns emerge. These findings suggest that radiating floral cue arrangements can evolve independently of specific perceptual biases by the pollinator except a preference for cues over a lack of cue. Our findings also suggest that radiating patterns can evolve without any associative learning by the pollinator.

Previous studies have suggested that receiver sensory biases may drive the evolution of floral guides. In Hymenopteran-selected displays, radiating guides may have evolved as a result of these receiver biases, whereby the receiver is exploited by the signaller (Naug & Arathi, 2007). The authors suggest that these receiver biases may have evolved in other behavioural contexts before the emergence of angiosperms, such as locating nest entrances, and led to the evolution of these patterns on flowers (Biesmeijer *et al.*, 2005). This would explain the apparent similarities in the visual stimuli of floral guides, stingless bee nest entrances and the patterning on insectivorous pitcher plants. However, our results suggest that such pre-existing receiver biases are not necessary for the evolution of radiating floral signals, which may be selected for purely through the time-related benefits to pollinators when used as guides. This is assuming that time benefits to the pollinator confer benefits to the plant through preferential visitation from pollinators (Waser & Price, 1985; Lehrer *et al.*, 1995), as well as increases to flower constancy and legitimate visits (Waser, 1986; Leonard *et al.*, 2013) and reduction of pollen loss (Harder & Thomson, 1989). Considering the parameters that are used within the genetic algorithm, the only pre-existing biases the pollinator needs are a preference for floral cues over a lack of cue. This also implies that associative learning is not necessarily needed for the evolution of radiating floral guides. However, associative learning and the evolution of floral guides are expected to be closely linked considering that such guides facilitate floral constancy which is of tremendous reproductive benefit to plants (Waser, 1986; Chittka, Thomson, & Waser, 1999).

The degree of floral guide radiation from the centre of the flower could be mediated by many factors, as demonstrated by our model. The strong positive correlation between mean cue connection value, an indicator for radiating lines, and the mean total cue count on a flower (Fig. 9), is not entirely surprising considering that the more cues a flower has the more they can be utilised into guides such as radiating lines. This is mirrored in the correlation between mean fitness and mean total cue count for size 21 flowers (Fig. 9). However, the relationship between cue connection value and the lonely cue count (*i.e.* the number of cues not connected to the nectary by other cues) was more complicated (Fig. 8). The negative correlation between cue connection value and the lonely cue count found in

larger flowers is probably due to flowers with higher numbers of lonely cues, where fewer of the cues are being implemented in radial patterns. This relationship is not seen in smaller flowers where the two outputs have a positive correlation, which is probably due to smaller flowers having lower degrees of radial patterns due to their size and the increased likelihood of the nectary being found by chance. If this is the case, additional cues which emerge through random mutations may be as likely to contribute to radiating patterns as they are to the number of lonely cues. It is important to note that lonely cues, which are not connected to the nectary, may still be arranged linearly and guide pollinators closer to the nectary without touching the nectary. This is exemplified in the 'dotted' or 'speckled' floral guides found in nature (Potgieter *et al.*, 1999; Goldblatt & Manning, 2009). However, the negative correlations between mean lonely cue count and mean fitness (Fig. 8) suggest that these lonely cues are detrimental to a pollinator's search for nectar.

The strong positive correlation between mean flower fitness and mean cue connection value, found in flowers of sizes 21 and 31 (Fig. 4), indicated that the higher the degree of radiating lines found on a flower, the higher the fitness of the flower. This increase in fitness is likely to be due to the time benefits afforded by the guides, as the measure of fitness is based on the number of movements a pollinator makes to reach the nectary. This is mirrored in other work where floral guides reduced both nectar discovery times in bees (Leonard *et al.*, 2013) and flower-to-flower flight times in bees and hummingbirds (Waser & Price, 1985). The negative correlation between these outputs in flowers of size 11 could be related to the fact that radiating lines are less useful on smaller flowers where there is a greater chance of finding the nectary by chance.

Although radiating cue arrangements developed in flowers with no degree of directionality (where directionality = 1, as seen in Fig. 3A), directionality still had a significant effect on the degree of radiating elements, with radiating elements and fitness increasing with the degree of directionality. This is potentially related an increased likelihood of radial patterns being used effectively in pollinators with higher directionality values, as flower visitors which move in a more linear fashion will be more able to 'follow' the guides compared to those with more erratic movements. Search strategies which involve straight or near-straight movements are also more effective (Zollner & Lima, 2017), which may contribute to the ubiquity of radiating floral guides. In addition to these increases in radiating elements at higher directionalities, later generations at lower directionalities tended to develop more rounded collections of cues around the nectary (Fig. 3A). These arrangements are reminiscent of the dark centres found on multiple floral species (Johnson & Dafni, 1998; Biesmeijer *et al.*, 2005; Van Kleunen *et al.*, 2007; Hempel de Ibarra & Vorobyev, 2009). Our results suggest that these dark centres may have evolved due to co-evolutionary relationships with pollinators which have lower degrees of linearity in their search strategies.

Increasing the distance a landing pollinator had to travel, through restricting its movement to cells sharing an edge (four-direction movement) or forcing it to land at the edge of the flower led to reduced fitness and increased cue connections (Figs. 7 and 3B). These results are reassuring, and imply that the landing locations of pollinators influences the arrangement of cues on the floral surface. This area-specific use of cues is seen in tubular flowers such a *Silene latifolia* where lilac compounds mark the flower entrance of the floral tube which guides flower visitors to the entrance and onwards to the nectary (Dötterl & Jürgens, 2005).

Floral size also influenced the degree of radiating elements on a flower and their fitness. Our results suggest that there are greater degrees of radiating patterns on larger flowers (Fig. 4). This makes sense, as that there is more room for the erroneous movement of pollinators on larger flowers and a higher likelihood to find the nectary by chance in smaller flowers. Small inconspicuous flowers have long been associated with self-fertilising species (Goodwillie *et al.*, 2010), but this is likely to relate to resource optimisation as self-fertilising species would have little use for floral guides. However, many small flowers do have patterns and guides (Daumer, 1958; Hempel de Ibarra & Vorobyev, 2009). Our results showing lesser degrees of radiating patterns on smaller flowers may relate to the fact that the metric used to measure the degree of radiating elements (cue connection value) may be unable to reach similar values in small flowers compared to larger flowers, even though these small flowers develop radiating elements. Floral guides on smaller flowers would still be beneficial to their visitors, who will need to orientate themselves within a flower. Floral guides on small flowers may also provide guidance cues as landing on these smaller flowers may be more difficult due to their size (Hempel de Ibarra, Langridge, & Vorobyev, 2015).

Radiating elements developed when pollinators had a preference for cues over blank cells (Fig. 3A), but did not develop when pollinators had no preference for cues (Fig. 3B). Considering that there was also no difference in cue connection value between simulations with preferences for cues (Fig. 7), this suggests that any preference for cues over a lack of cues is sufficient for the development of radiating elements and that this preference is necessary for this development. However, as mean fitness did increase with cue preference value (Fig. 7), it is possible that the preference for cues over blanks allows pollinators to better utilise the radiating guides. This highlights the importance of the physiological limitations, neuro-sensory filters and abilities to make use of sensory information which shape preferences and create contrasts between different floral organs and locations (Lunau & Maier, 1995; Dobson, Groth, & Bergström, 1996; Leonard & Papaj, 2011). However, as this model does not fully explore the perceptual capabilities of flower visitors, more experimental work is needed.

When using tools such as genetic algorithms to gain insights into areas as complex as the evolution of floral displays we must be keenly aware of the limitations faced. Within the genetic algorithm used

there have been several aspects which were considered outside the scope of this project, but may highlight opportunities for future research. The two-dimensional square flowers are highly simplified, and real flowers have complex three-dimensional morphological characteristics where the very shape of the flower influences flower visitor movement (Laverty, 1994). Future genetic algorithms which integrate more complex floral structures, and the planes on which they are presented (Wolf, Roper, & Chittka, 2015), could give insights into the evolution of cue arrangement on a wide range of flowers. The current model also uses a single floral cue with characteristics of both visual and olfactory signals for use by pollinators to locate the nectary whereas flowers use pigments, scents, surface textures and the three-dimensional structure of the flower for orientation (Kevan, 1978; Kevan & Lane, 1985; Dobson, 1987; Heinrich, 2004; Raguso, 2004a; Lawson *et al.*, 2017a). Each of these cues has different effects at different scales compared to those used in the current genetic algorithm where cues must be within the surrounding cells to elicit a behavioural response. For instance, scented nectars can inform pollinators of reward availability and surface textures can orientate pollinators in a particular direction (Kevan & Lane, 1985; Raguso, 2004b). Within the current genetic algorithm, these cues are each coded for by singular genes, which is unlikely to be seen in nature where the regulation of pigment biosynthesis is often less specific (Shang *et al.*, 2011). Scent and pigments, as well as other signals, can also inform and attract flower visitors before landing (Jakobsen & Olsen, 1994; Giurfa *et al.*, 1995; Wright & Schiestl, 2009), but this pre-landing information is not part of the current genetic algorithm. Future genetic algorithms could incorporate multiple cues and gradients of cues (Lunau, 1993; Bergström, Dobson, & Groth, 1995) rather than the simplified 'on/off' system used here, as floral displays often have multiple aspects within singular modalities, such as multiple colours and scents (Bergström *et al.*, 1995; Dafni & Kevan, 1996; Warren & Mackenzie, 2001; Lawson, Whitney, & Rands, 2017b). The metabolic costs of cues which are seen in pigment and volatile production could also be explored in future simulations (Paré & Tumlinson, 1999; Brockington *et al.*, 2011).

The genetic algorithm used did not incorporate any innate preferences other than a preference for cues over a lack of cue, whereas pollinators are known to have many innate preferences in terms of colours and scents which influence which flowers are visited while foraging (Giurfa *et al.*, 1995; Lunau & Maier, 1995; Lunau, Wacht, & Chittka, 1996; Riffell *et al.*, 2008). One of these preferences relates to the symmetry of flower shapes and their floral guides as pollinators are known to have a preference for symmetrical flowers and assess the degree of symmetry in flower patterns before landing (Lehrer *et al.*, 1995; Møller, 1995). Flowers are also thought to have been bilaterally symmetrical early on in the evolutionary history (Marazzi *et al.*, 2006), which suggests that incorporating this preference for symmetry into future models may be worthwhile. Particularly since floral signals are more likely to be selected for when they exploit the perceptual systems and preferences of effective or abundant pollinators (Dyer *et al.*, 2012). Radiating lines themselves have also been shown to increase the attractiveness of flowers compared to flowers without these linear elements (Manning, 1956; Free,

1970; Dinkel & Lunau, 2001; Leonard & Papaj, 2011; Leonard *et al.*, 2013). These innate sensory preferences of pollinators can be overcome or altered through associative learning (Hammer & Menzel, 1995; Giurfa, 2007) which could also be explored in future simulations.

Floral displays are thought to play important roles in the evolutionary diversification of plants and their pollinators, making the evolution of cue arrangements a compelling area of study. Within this study, we have modelled one of many possibilities, which suggests that pollinator directionality, starting location, movement type and flower size influence the development of radiating guides on flowers. Our findings imply that that pre-existing receiver biases, preferences or associative learning are not necessary for the evolution of radiating floral guides in simulated flowers, other than a preference for a stimulus over the absence of one. However, as this model does not fully explore the full extent of the interactions between plants and their pollinators, it is our hope that this study motivates more empirical studies in this area.

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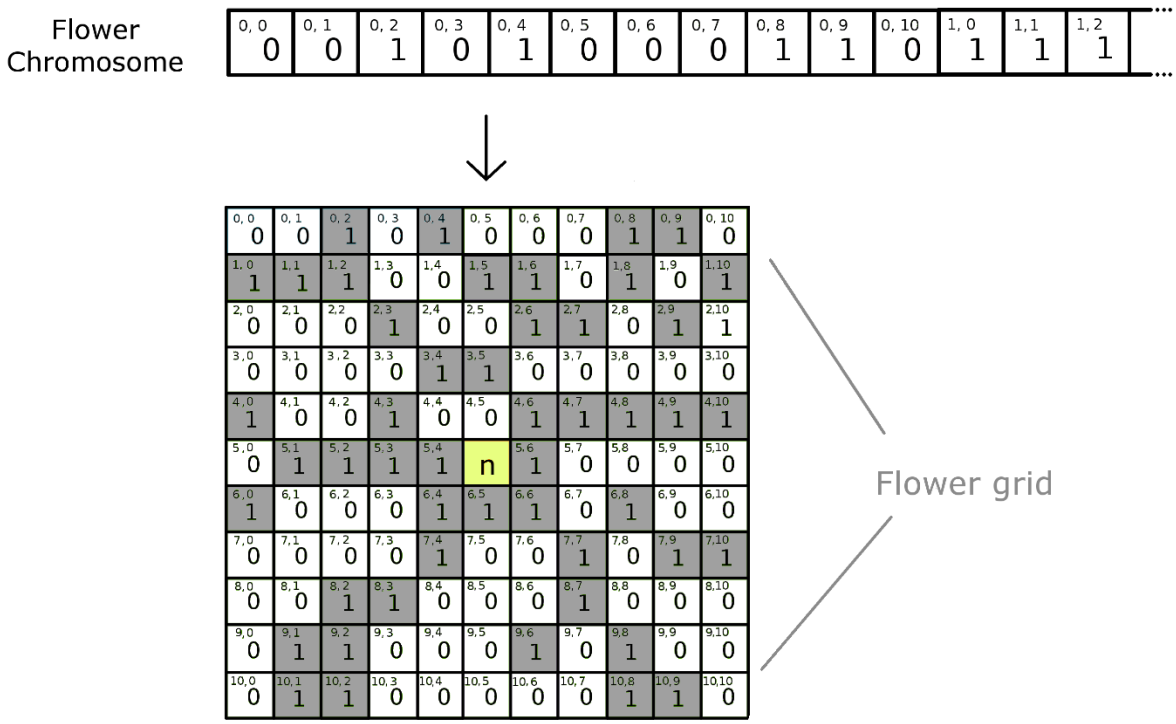

Figure 1. Diagram of the flower chromosome and flower grid. Grey ‘1’ cells represent cells with cues and white ‘0’ cells represent cells without cues. The central ‘n’ cell represents the nectary.

0	0	0	0	x	0	0	0	6	0	0
0	6	0	0	0	0	0	5	5	0	0
0	0	5	5	0	0	0	4	0	0	0
0	0	0	4	0	0	3	0	0	0	x
x	0	0	3	2	2	0	0	0	0	0
0	x	0	0	2	1	2	3	4	0	0
0	0	0	0	2	0	0	3	4	5	6
0	5	4	3	3	0	0	0	0	0	0
0	0	0	4	0	0	0	x	0	0	0
0	0	5	0	0	0	0	x	x	0	0
0	0	6	0	0	x	0	0	0	x	0

Figure 2. Diagram of the cue connection values on an individual flower. The '1' represents the nectary cell, other numbered cells represent cells with cues and display the cue connection value of that individual cell, zeros represent blank cells and x's represent cues which are not connected to the nectary via other cues which contribute to the 'lonely cue count'.

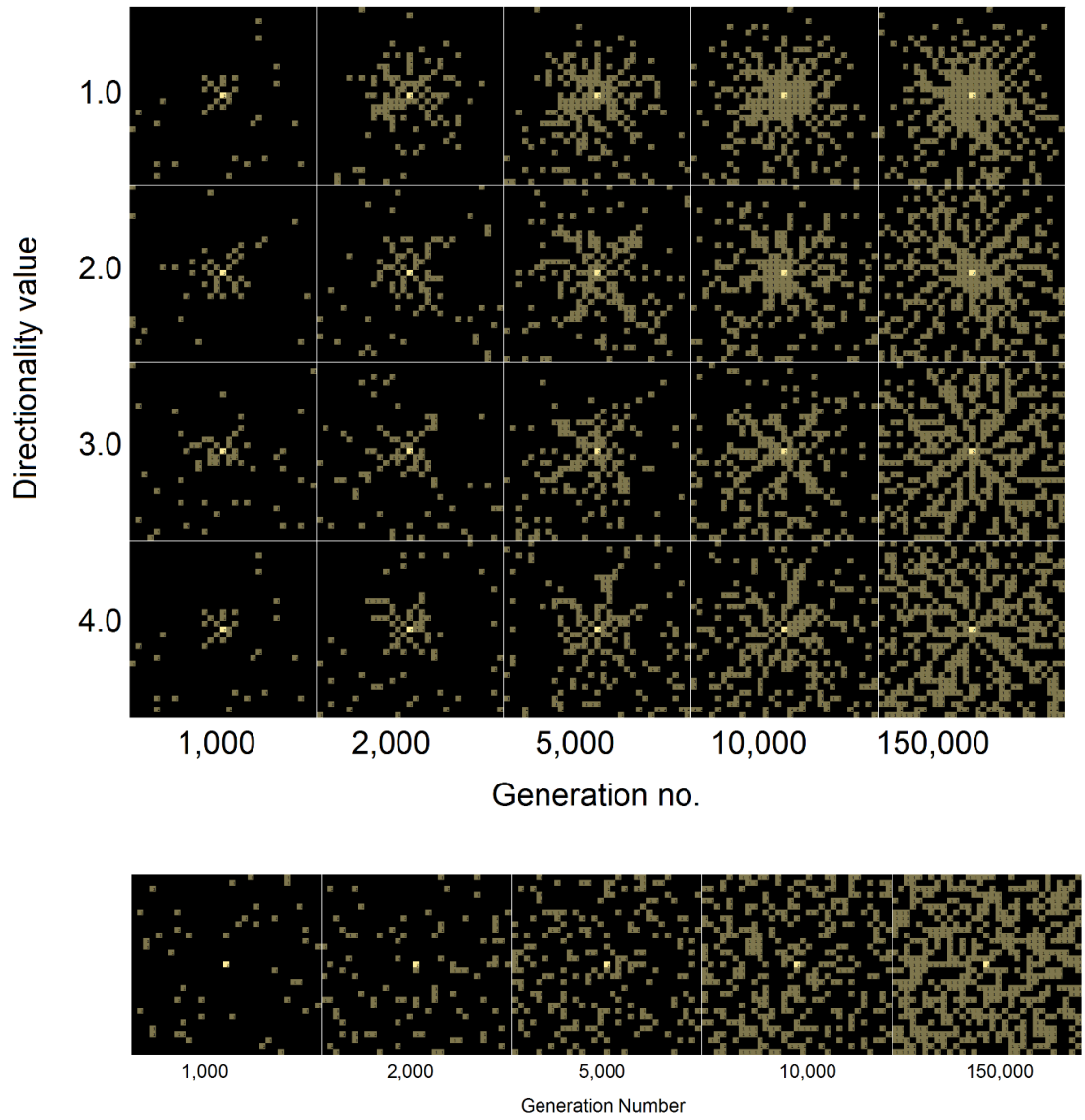

Figure 3. Example of floral cue pattern evolution. (A) Outputs displaying sample flower from five generations of four directionality values. Black space represents blank flower cells, beige areas represent cue cells and the yellow in the centre of each flower represents the nectary. Early generations show the development of guides with later generations showing radiating guides. For pollinators with a directionality value of one, there is an equal likelihood that the pollinator will move in any direction. Increases from this directionality value increase the likelihood of moving forward compared to the other seven directions. The diagram displays outputs from flowers with 8-way movements starting from the edge, cue preference values of 6, and size 31 flowers. (B) Outputs of sample flower demonstrating the cue arrangements of flowers with a cue preference value of 1. At a cue preference value of one, pollinators do not have any degree of preference for cues over blank cells. No development of radiating guides occurs across generations. Example is taken from flowers with 8-way movements starting from the edge, cue preference values of 1, and size 31 flowers.

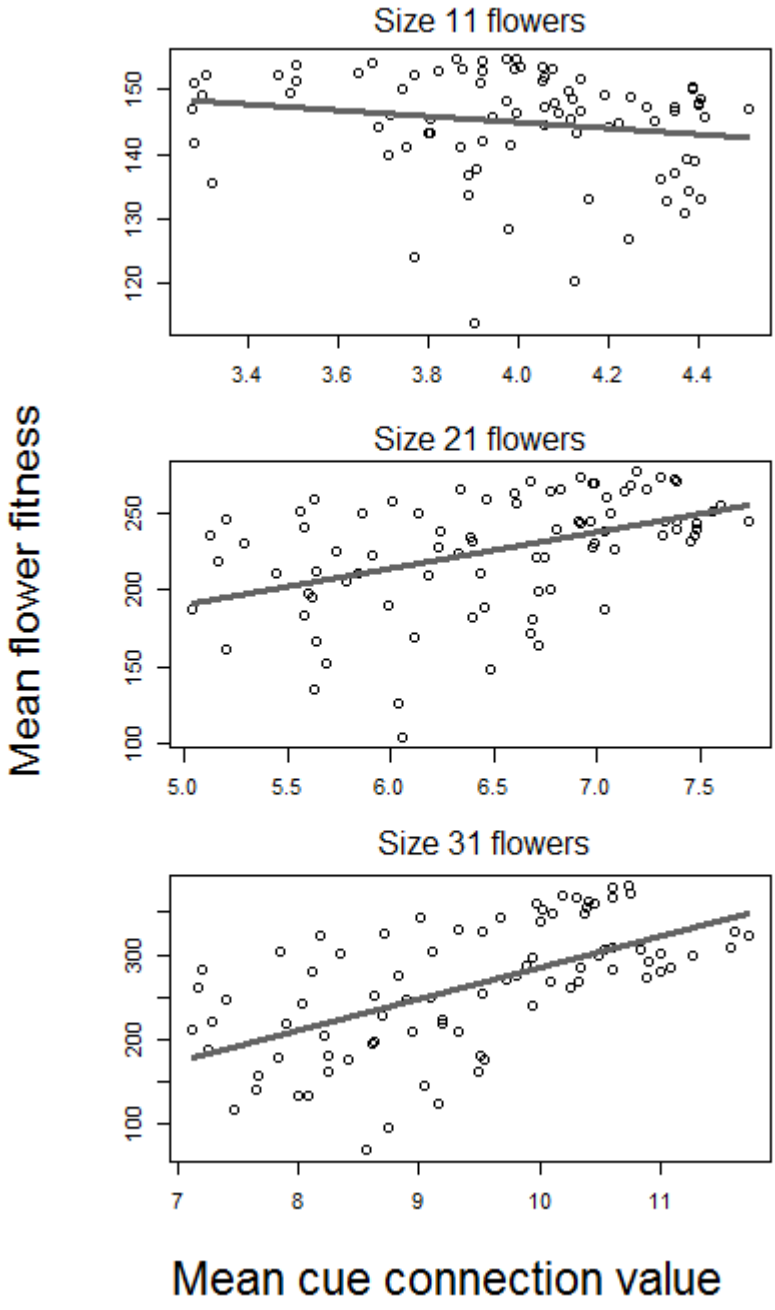

Figure 4. The relationship between mean cue connection value and mean fitness of sample flowers across three flower sizes. Data points represent the average values of 100 sample flowers from a population of 1000 over 150,000 generations in total.

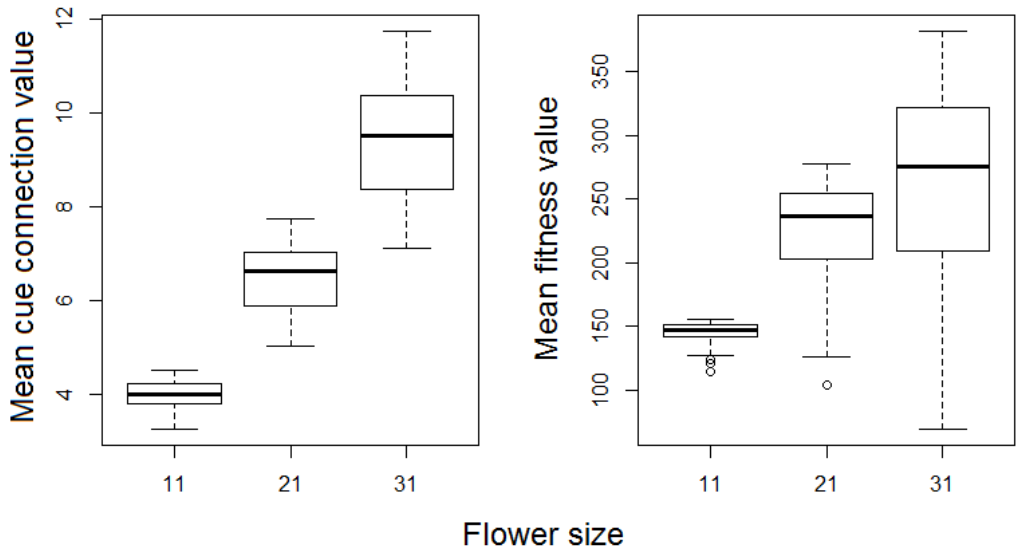

Figure 5. Boxplots displaying median, interquartile ranges and upper and lower quartile ranges of data of the relationship between cue connection value, fitness and flower size. This demonstrates how larger flowers can develop more radiating guides and higher fitness values as there is more space for these guides to develop. Pairwise *post hoc* comparisons show that all means in both panels differ from each other with $p < 0.001$.

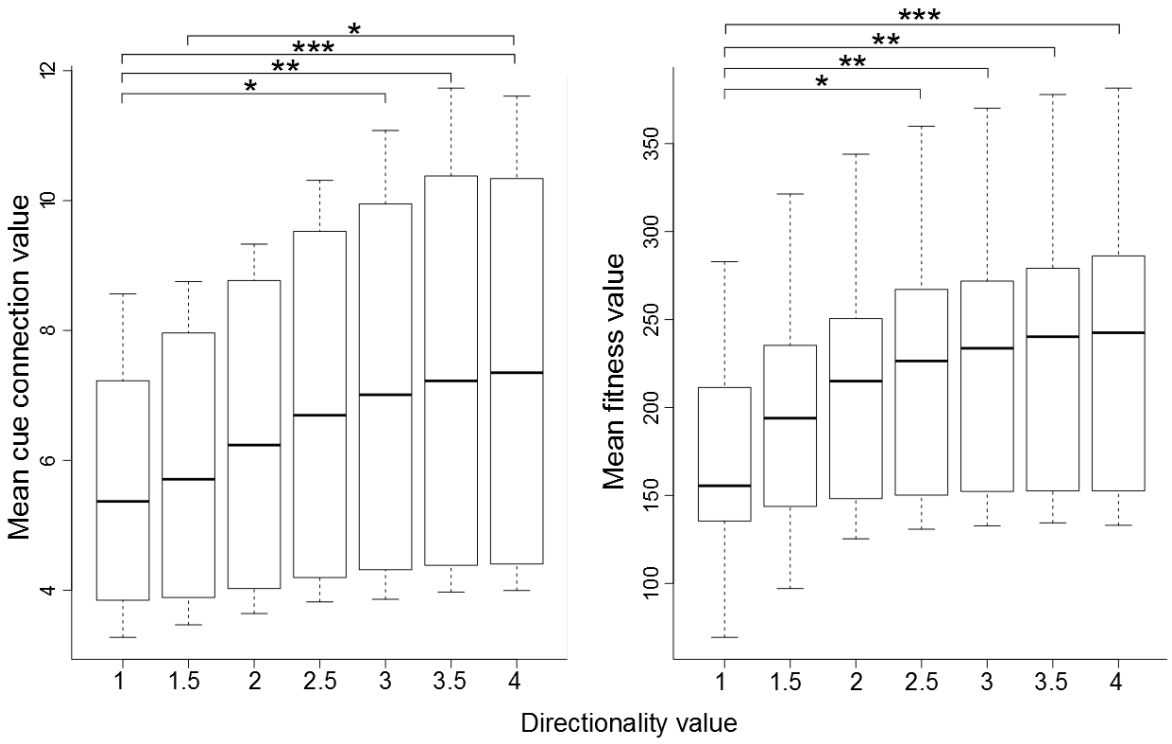

Figure 6. Effect of pollinator movement directionality on cue connection and fitness. See Fig. 5 for details of the boxplot. Significant pairwise *post hoc* comparisons are shown, where * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

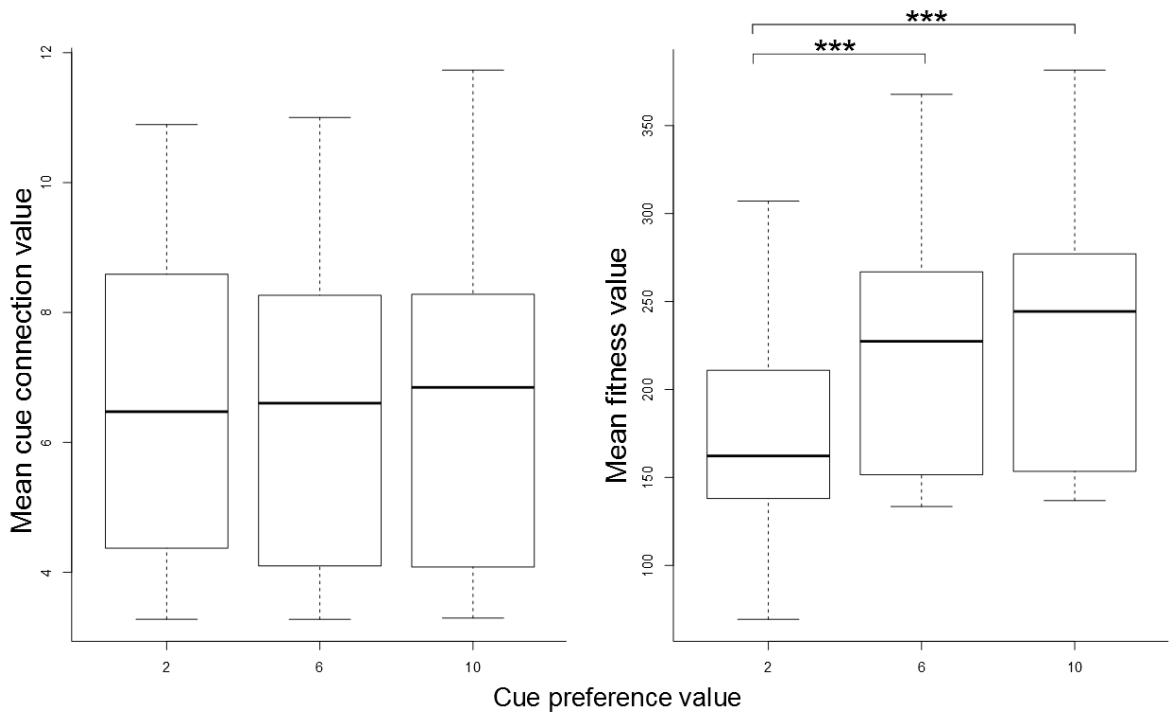

Figure 7. The relationship between mean cue connection value over three cue preference values of pollinator. Cue preference value being the probability that a pollinator will move to a cue over a blank cell. No difference was found in cue connection values between groups with different cue preference values. Significant paired *post hoc* comparisons are shown, where *** $p < 0.001$.

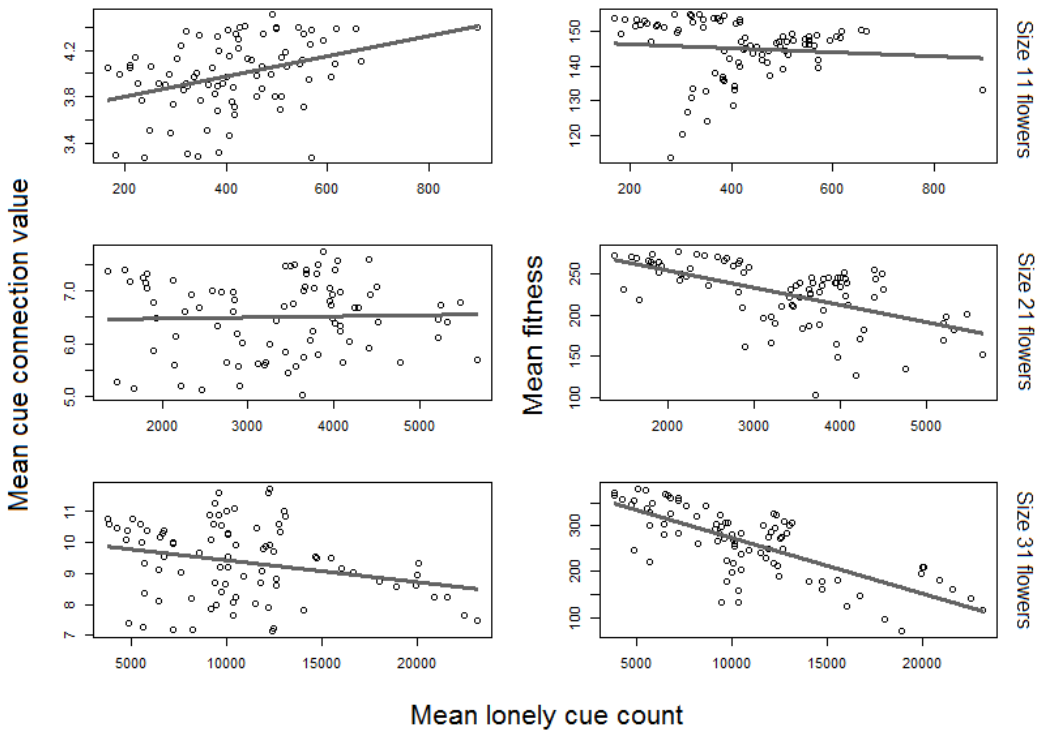

Figure 8. The relationship between mean cue connection value and mean lonely cue count and fitness and mean lonely cue count across three flower sizes.

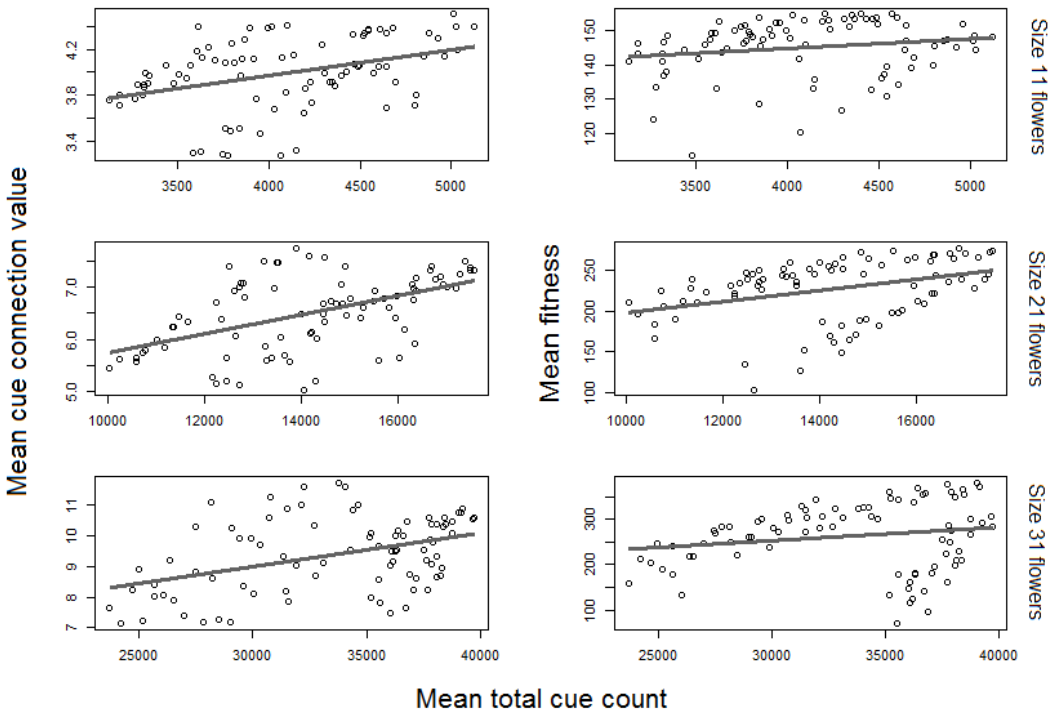

Figure 9. The relationship between mean cue connection value and mean total cue count and fitness and mean total cue count across three flower sizes.